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Synthesis and NMR Study of Two Lipophilic Iron(III) Sequestering Agents Based on 8-Hydroxyquinoline; H-bonding and Conformational Changes

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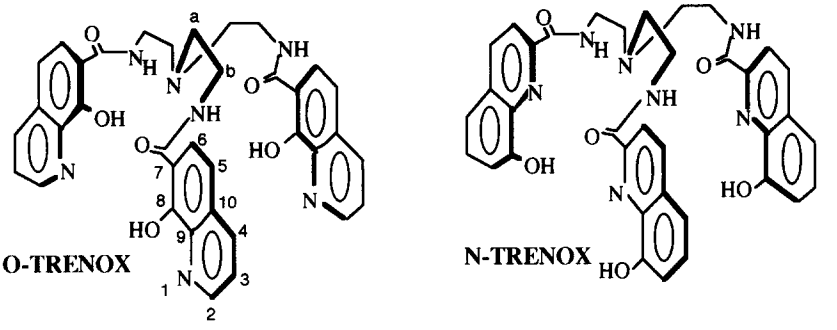
Abstract - The synthesis of two tripodal iron chelating agents based on 8-hydroxyquinoline is described. The ligands consist of tris(2-aminoethylamine) (spacer) linked in 2- or 7- position to three 8-hydroxyquinoline units (allowing the complexation of iron). NMR study of these ligands in DMSO-d_6 solutions evidence intramolecular H-bond networks inducing conformational changes in relation to the protonation state of the tertiary amine.

INTRODUCTION

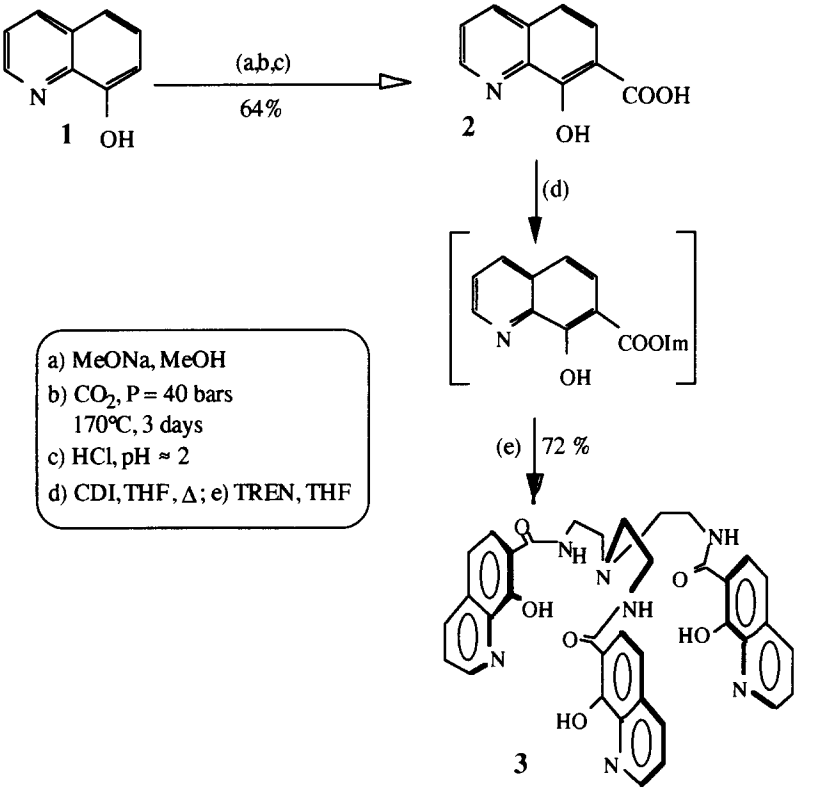
There is interest in natural (siderophores) or synthetic iron (III) sequestering agents which are (or may be) involved in iron transport in microorganisms or plants and which can be used for therapeutical or agrochemical applications[1]. Shanzer et al. have shown that iron complexing abilities of siderophores are widely governed by hydrogen-bonding and preorganization of the ligand[2]. Hydrogen bonding in catechoylamides has been extensively studied by Raymond et al.[3]. On the other hand, the transport of siderophores is related to their structure and strongly depends on the favored conformations for the metal complex and membrane proteins. Nevertheless, most of the studies on natural or abiotic siderophores have been focused on the iron complexing abilities; relatively little attention has been paid to the structure of the free ligands in solution. Some papers describe the conformational behavior of free ligands [2-9].

In this paper, we describe the synthesis and the solution studies of two related tripodal iron sequestering agents (O-Trenox and N-Trenox, depicted in Figure 1) which contain 8-hydroxyquinoline (oxine) subunits. The iron (III) complex of the sulfonated derivative of O-Trenox has been shown to act as a powerful agent for plant nutrition and treatment of iron chlorosis[10]. Intramolecular hydrogen bonding in these ligands is essentially depending on the protonation state of the coordination sites (pyridine nitrogen and oxygen atoms) and of the tertiary amine. An NMR study has been carried out in order to obtain information on the possible conformations of the ligand molecule since the pH-dependent chemical shifts of nuclei in the vicinity of the proton binding sites reflect their protonation state.

Figure 1 : O-Trenox and N-Trenox



Scheme 1 : synthesis of podand O-Trenox



RESULTS AND DISCUSSION

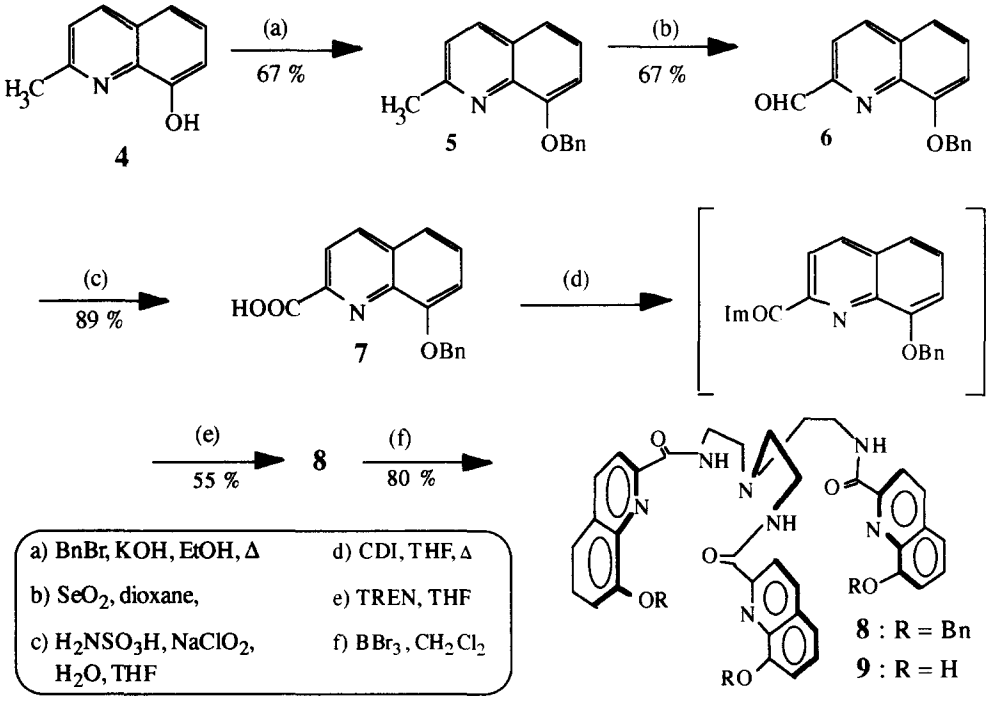
Synthesis of 8-quinolinolate tripodal ligands

The synthetic strategy is centered on the use of 8-hydroxyquinolinyl subunits containing, either in position 2 or in position 7, carboxylate moieties which are then activated and then conjugated with a tripodal tetraamine [tris(2-aminoethylamine) = TREN].

The activated and (if necessary) protected 8-quinolinolate arms were obtained by the conventional means outlined in Schemes 1 (series "7") and 2 (series "2"). Coupling of the carboxylate moieties was most conveniently accomplished using N,N'-carbonyldiimidazole[11].

No description of the synthesis of the podand **3** (series "7") has been reported in the literature. The podand **9** (series "2") has been prepared by Shrader et al.[12]. We have developed a different synthetic pathway, involving 8-hydroxyquinaldine (protection of OH and oxidation of CH_3).

Scheme 2 : synthesis of podand N-Trenox



NMR studies

The two ligands contain seven acido-basic sites : three pyridine nitrogens, one tertiary nitrogen and three hydroxyl protons. The pK_a in aqueous solution of the analogous sulfonated molecules (sulfonation on the 5 position of the quinoline ring) will be described elsewhere[13]. The values are in the range 2-5 for pyridines, 7-9 for hydroxyl groups and 6-7 for tertiary amine. The deprotonation of the tertiary nitrogen was studied by NMR techniques in DMSO d_6 solutions. In the ligands 3 and 9 the three arms are equivalent since one NMR signal is usually observed for each proton and carbon. This observation suggests the presence of C_3 symmetry conformation or of rapidly interconverting conformations. As we already showed in another series of iron tripodal chelators[14], the deprotonation of the tertiary amine has been found to result in large chemical shifts effects of the methylene protons a and b (Fig.1) : $\Delta\delta$ are about -0.8 and -0.3 ppm for the protonated and the deprotonated forms respectively. In the pH range studied, the pyridine nitrogens are deprotonated.

By studying the tertiary amine deprotonation on raising the pH of the solution, the change of the ^1H NMR spectrum of O-Trenox revealed a classical behaviour. Indeed, the spectra showed a continuous variation of the chemical shift of the methylene protons from $\delta_a = 3.72$ and $\delta_b = 3.94$ ppm to $\delta_a = 2.80$ and $\delta_b = 3.59$ ppm, indicating a fast exchange in solution between the protonated and the deprotonated form of the ligand.

On the contrary, the deprotonation of the tertiary amine in N-Trenox (DMSO d_6 solutions) resulted in an unusual effect as shown by the ^1H NMR study. The spectra recorded at various pH exhibited for intermediate pH two sets of signals for the methylene protons a and b (fig 2b) The chemical shifts $\delta_a = 3.74$ and $\delta_b = 3.96$ ppm correspond to the signals of the protonated amine form (fig 2a) and the values $\delta_a = 2.93$ and $\delta_b = 3.59$ ppm correspond to the signals of the deprotonated amine form (fig 2c). These values are very close to those observed with O-Trenox for the protonated and deprotonated species respectively. The aromatic proton H-3 was also splitted ($\Delta\delta = 0.1$ ppm). The same splitting was observed for the ^{13}C signals (fig 3b) of the methylene carbons ($\Delta\delta_a = 1.8$, $\Delta\delta_b = 3.6$ ppm), of the aromatic carbon C-2 ($\Delta\delta = 0.7$ ppm) and of the carbonyl carbons ($\Delta\delta = 0.9$ ppm). The pH dependence of the ^1H NMR spectra was also studied for the O-benzylated derivative of N-Trenox. The splitting was observed for the aromatic H-4 protons and for the methylene protons of the benzyl group at room temperature (fig 4). When warming the sample, the splitted signals coalesced at 87°C. According to these observations, it can be concluded that the two species (protonated and deprotonated tertiary nitrogen) of N-Trenox and of its O-benzylated derivative are in slow exchange at the NMR time scale.

The exchangeable protons were studied in the ^1H NMR spectra of N-Trenox . The signal at 10.6 ppm was assigned to the proton on the tertiary nitrogen and disappeared on raising the pH (Fig 2 b-c). The hydroxyl protons were observed as a narrow signal (10.0 ppm in Fig 2a and 10.1 ppm in Fig 2c). The N-H amide proton signal shifted from 10.1 ppm (protonated tertiary amine) to 9.7 ppm (deprotonated tertiary amine). It should be emphasized that the signals of the N-H amide proton and of the hydroxyl proton are narrow indicating no chemical exchange for these protons. This suggests that they are involved in hydrogen bond. It should be noted that when ^1H NMR studies were performed in DMSO d_6 - D_2O mixtures (1/1, v/v), no splitting of the signals was observed.

These results indicated that the relative stability of the tertiary amine protonated and deprotonated forms of N-Trenox could be due to intramolecular hydrogen bonding. These could induce stabilization of different conformations of N-Trenox according to its protonation state. NOE experiments were carried out in order to gain more information on the possible conformations of the ligands.The NOE difference spectra for the

Figure 2 - 400 MHz ^{1}H NMR spectra of N-Trenox (a) in acidic (protonated tertiary N form), (b) neutral (mixture of protonated and deprotonated tertiary N forms), (c) basic (deprotonated tertiary N form) DMSO-d_6 solutions (see numerotation in Figure 1).

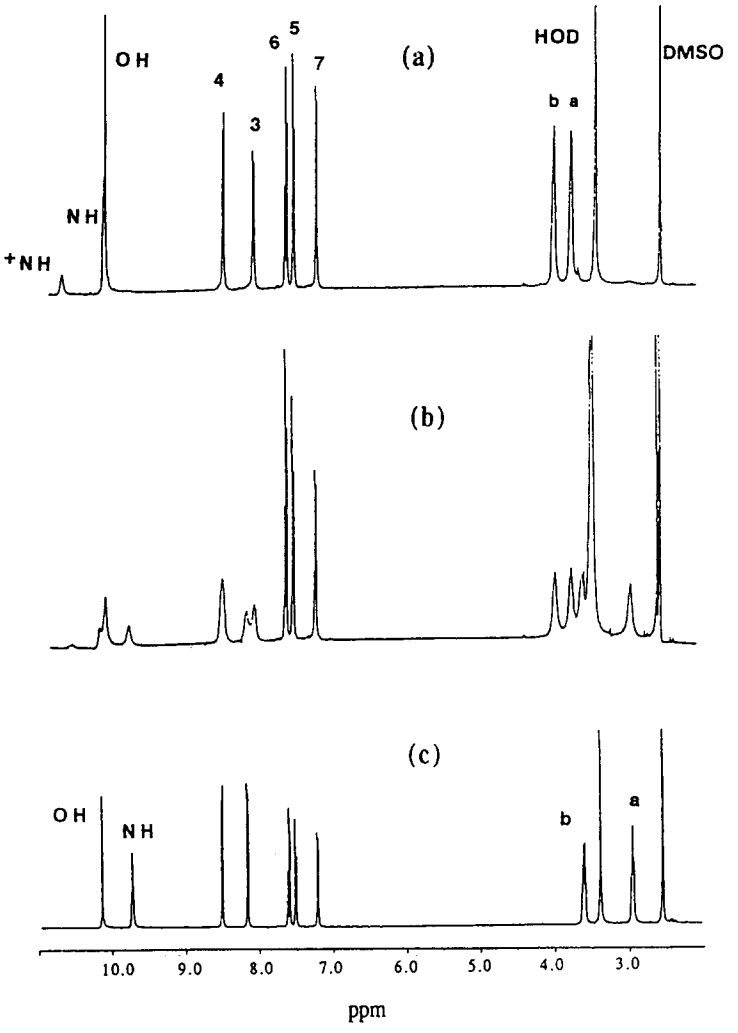


Figure 3 - 100 MHz ^{13}C NMR spectra of N-Trenox (a) in acidic, (b) neutral, (c) basic DMSO-d_6 solutions (see numerotation in Figure 1).

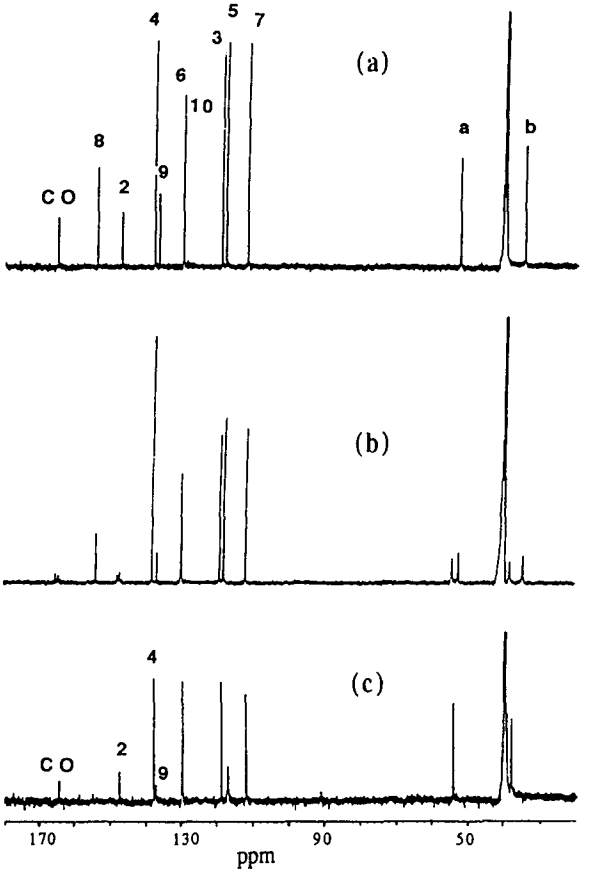
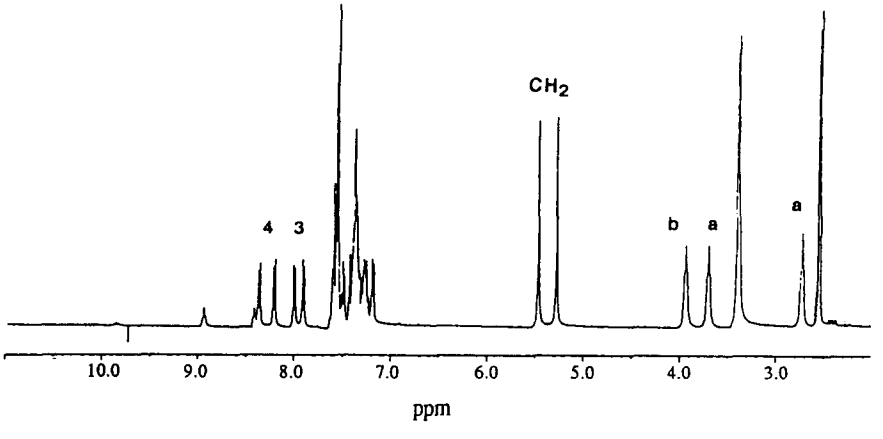


Figure 4 - 400 MHz ^1H NMR spectra of O-benzylated N-Trenox in neutral (mixture of protonated and deprotonated tertiary N forms) DMSO-d_6 solutions (see numerotation in Figure 1).



protonated and the deprotonated forms of the O-Trenox and of the N-Trenox were obtained by irradiating the N-H amide proton. The irradiation resulted in NOE enhancement of H-3 in the protonated N-Trenox (Fig 5a), of O-H in the deprotonated N-Trenox (Fig 5b) and H-6 in O-Trenox (Fig 6b-c). The NOE enhancement data clearly suggest that (i) for N-Trenox the N-H amide is close to H-3 in the protonated form and to O-H in the deprotonated form (ii) for O-Trenox the N-H is close to H-6 whatever the protonation state of the tertiary amine. These last results added to the preceeding observations led us to propose the conformations for O-Trenox and N-Trenox depending on the protonation of the tertiary amine (Fig. 7).

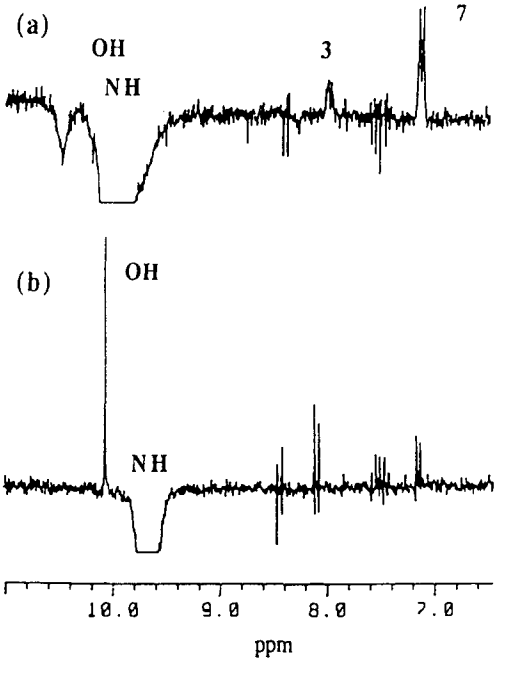


Figure 5 - NOE difference spectra of N-Trenox by irradiating the amide NH proton (a) protonated tertiary N form (b) deprotonated tertiary N form.

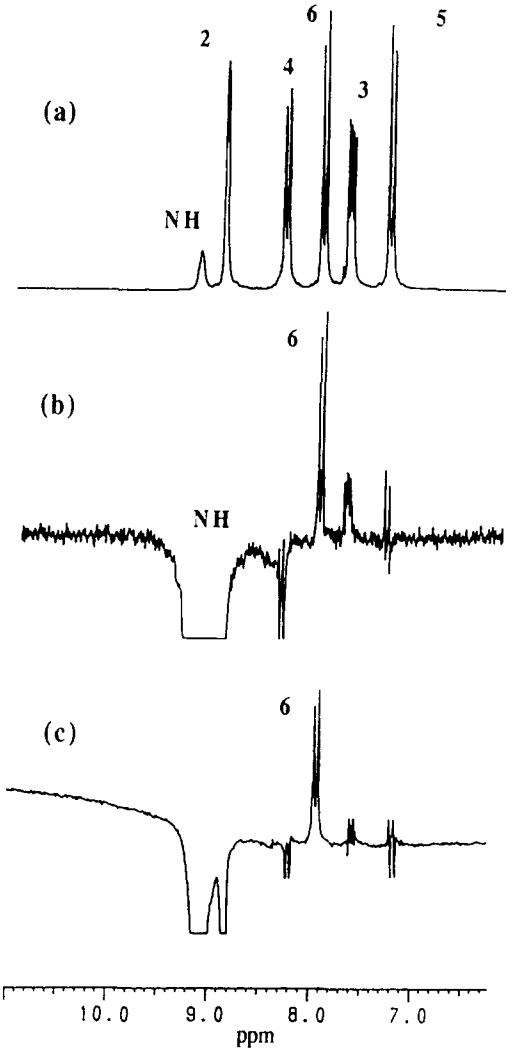


Figure 6 - NOE difference spectra of O-Trenox (a) non irradiated spectrum, (b) and (c) irradiation of the amide NH proton, protonated tertiary N and deprotonated tertiary N forms respectively

When the tertiary amine of N-Trenox is protonated, an hydrogen bond is assumed to be established between this proton and the carbonyl oxygen of each arm of the molecule (Fig.7A). Phenol proton can also be involved in the hydrogen bond network with the pyridine nitrogen thus favouring the conformation A. When the tertiary amine is deprotonated, the hydrogen bond NH...O=C is broken and a new favourable network of H-bond is established between the amide proton and both the tertiary amine and the pyridine nitrogen (Fig. 7B). The conformation B is consistent with the NOE measured between NH and OH. These H-bonds induce the formation of three five-membered rings for each arm of the ligand leading to a probably very stable folded conformation. The tertiary amine deprotonation implies a rotation around the carbonyl - quinoline bond. According to the ^{1}H and ^{13}C NMR studies, this rotation is found to be slow at room temperature on the NMR time scale. For the two conformations, the spatial environments of the aromatic H-3 and hydroxyl protons (or methylene and aromatic protons in the o-benzylated derivative) are different, which explains the chemical shifts variations.

In the case of O-Trenox (Fig.7C,D), there is no need of rotation around the carbonyl - quinoline bond when the deprotonation occurs. Indeed, the most favourable H-bond is established between phenol proton and carbonyl oxygen whatever the protonation state of the tertiary nitrogen. The aromatic protons H6 and H5 have always the same spatial environment so their chemical shift do not vary. The amide protons are assumed to be hydrogen bonded to the tertiary amine in the deprotonated form as generally expected for this type of tripodal structure[15,16].

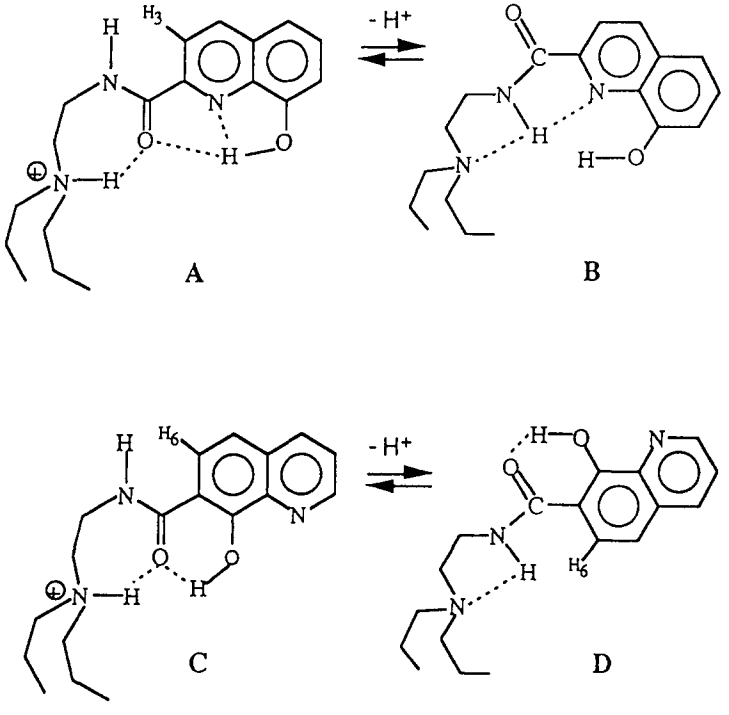


Figure 7 - The two possible conformations of N-Trenox (A, B) and of O-Trenox (C, D).

A possibility of inter-strand H-bond network might be envisioned on the basis of molecular models. But the same behavior was observed for the O-benzylated derivative of N-Trenox for which the formation of inter-strand H-bonds is hindered by the benzyl group. We are led to suggest that the only intra-strand H-bonding has to take into account to explain the NMR results. Furthermore, the solvent DMSO is unfavorable to intermolecular and/or inter-strand H-bonds.

The presence of intramolecular hydrogen bond networks in the free ligand is an element of preorganization to provide efficient iron(III) chelation as it was claimed to explain the potency of the natural enterobactin siderophore[2]. Upon complexation, a rearrangement of the hydrogen bonds must occur when passing from the free to the coordinated ligand in order to stabilize the complex. The structural features of the ligand must hence favor the change of conformation. Furthermore, coordination with Fe(III) implies deprotonation of the basic sites of the ligand (hydroxyl groups, tertiary amine). Our results show that the rearrangement of the hydrogen bond network in O-Trenox occurs without constraint. On the contrary, the change of conformation in N-Trenox appears to be hindered resulting from a conformation (Fig. 7B) stabilized by an extended hydrogen bond network with five-membered rings. Since iron binding results in rearranged hydrogen bonds, it can be presumed a better binding efficiency for O-Trenox than for N-Trenox. Metal binding studies confirm the better ability of O-Trenox to complex iron(III). Preliminary results concerning the iron chelating properties of the sulfonated derivatives of these ligands have been reported elsewhere[20].

EXPERIMENTAL SECTION

Materials and Equipment

Solvents were purified by usual techniques. Boron tribromide (Aldrich) was vacuum distilled and stored under Argon. The amine TREN was distilled from sodium. All other compounds used were of reagent grade and were not further purified.

Spectra were collected on a Perkin Elmer spectrometer (IR) and on a NERMAG R 10 10 C (Mass spectra).

Microanalyses were performed by the Analytical Services Laboratory, CNRS, Solaise (France).

Melting points were taken on a Büchi apparatus and are uncorrected.

NMR measurements

NMR - ^{1}H and ^{13}C measurements were carried out in 5 mm tubes at 25°C with a Bruker AC200 or a Bruker AM 400 operating at 200.13 and 400.13 MHz respectively for ^{1}H and 50.31 and 100.62 for ^{13}C. Sodium 3-trimethylsilyl-propane-1-sulfonate (TSP, Merck) was used as an internal reference. The ligand concentration was 0.005 mol dm^{-3} in DMSO d_6 solutions and the pH was adjusted with adding DCl or NaOD. The ^{1}H-^{1}H NOE spectra were obtained using the standard sequence.

Signal assignments : pyridine ring protons and phenol ring protons were assigned using homonuclear decoupling and on the basis of shielding effect of the hydroxyl group in ortho position. A heteronuclear ^{13}C-^{1}H shift correlation experiment was performed to assign the ^{13}C signals : sweep width of 7000 Hz (2K data points) in f_2 (^{13}C) and ± 800 Hz (128 t_1 values zerofilled to 1K) in f_1 (^{1}H). In the f_2 dimension (^{13}C) a 2 Hz

exponential line broadening was used prior to Fourier transformation whereas in the f_1 dimension (^1H) a -0.1 Hz exponential line broadening and a 0.1 Hz gaussian multiplication were used.

7-Carboxy-8-hydroxyquinoline : 2

8-hydroxyquinoline **1** (5 g, 34.00 mmol) was added to a solution of sodium methanolate (sodium 1g, 43.00 mmol, methanol 50 cm^3) ; the mixture was stirred for 15 min., then methanol was evaporated yielding the sodium salt as a yellow solid. The sodium salt was thoroughly dried under vacuum and then treated in autoclave by CO_2 under pressure (40 bar) at 170°C for 3 days. The resulting mixture was taken with water, filtered and acidified to precipitate crude **2.** The product was purified by recrystallization from water (4.1 g, 21.7 mmol, 64%).

Tf = 248°C; (lit[17] 237-50°C)

IR (KBr) 3500-3100 cm^{-1} υ(O-H) phenol ; 3070-2300 υ(Csp^2-H) et υ(O-H) acid ; 1655 cm^{-1} υ(C=0) ; 1625-1590-1450 cm^{-1} υ(C=C).

^1H RMN ($CDCl_3$+DMSO d_6, 200 MHz): δ 7.20 (d, J=8.5 Hz, 1H, H_6); 7.53 (dd, J_1=8.2 Hz, J_2=4.2 Hz, 1H, H_3); 7.65 (d, J=8.7 Hz, 1H, H_5); 8.19 (dd, J_1=8.2 Hz, J_2=1.1 Hz, 1H, H_4); 8.83 (dd, J_1= 4.2 Hz, J_2=1.1 Hz, 1H, H_2)

^{13}C RMN ($CDCl_3$+DMSO d_6, 200 MHz) : δ 114.17, 123.75, 127.68, 139.70 (CH cycl.) ; 111.73, 132.16, 136.61 ($C_{quat.cycl.}$) ; 146.40 (C_2=N) ; 160.83 (C_8-OH) ; 170.92 (COOH).

Tris-N-(2-aminoethyl-[8-hydroxyquinoline-7-carboxamido]) amine : **3 (O-Trenox)**

2 (0.50 g, 2.64 mmol) in 50 cm^3 of freshly purified THF was treated with 1,1'-carbonyldiimidazole (0.47 g, 2.91 mmol) under reflux for 1 hour, then TREN (0.13 g, 0.88 mmol) in 10 cm^3 THF was added dropwise and the mixture was stirred overnight under reflux. The solvent was evaporated under vacuum and the residue was taken up with 100 cm^3 of $CHCl_3$ and the solution was successively washed with aqueous ammonium chloride and brine and dried on $MgSO_4$. Evaporation gave crystals which were recrystallized in methanol-water (0.42 g, 0.64 mmol, 72%).

Fp = 101°C; IR(KBr) 3500-2970 cm^{-1} υ(O-H), υ(N-H), υ(Csp^2-H) ; 1620 cm^{-1} υ(C=O); 1610-1550-1461 cm^{-1} υ(C=C)

^1H NMR (DMSO d_6, 400 MHz): δ 2.87 (br s, 6H, CH_2); 3.55 (dt, J=5.6 Hz, 6H, CH_2); 7.20 (d_{AB}, J_{AB}=8.8 Hz, 3H, H_6); 7.56 (dd, J_1=8.3 Hz, J_2=4.1 Hz, 3H, H_3); 7.87 (d_{AB}, J_{AB}=8.8 Hz, 3H, H_5); 8.19 (dd, J_1=8.3 Hz, J_2=1.4 Hz, 3H, H_4); 8.79 (dd, J_1=4.1 Hz, J_2=1.4 Hz, 3H, H_2); 8.86 (t., J≈5.0 Hz, 3H, NH)

^{13}C NMR (acetone d_6+DMSO d_6, 200 MHz, 2D "Delta hetero"): δ 38.71 (CH_2); 54.32 (CH_2); 117.62 (C_6), 123.87 (C_3), 126.74 (C_5), 136.55 (C_4); 114.11, 131.01, 139.70 ($C_{quat.cycl.}$); 149.22 (C_2); 155.50 (C_8-OH); 167.88 (C=O).

FAB^+ MS (NBA): $(M+H)^+$ 660, 515, 489, 471, 458, 446, 215, 172

FAB^- MS (NBA): $(M-H)^-$ 658, 513, 487, 470, 458, 444

HRMS FAB(+): calcd for $C_{36}H_{34}N_7O_6$ $(M+H)^+$ 660.2570; found 660.2579

Anal. : Calcd (Found) for $C_{36}H_{33}N_7O_6$, HCl, H_2O: C, 60.57 (60.89); H, 5.09 (5.02); N, 13.74 (13.30)

2-Methyl-8-benzyloxyquinoline : **5**[18,19]

8-hydroxyquinaldine **4** (20.00 g, 125.64 mmol) was added to a solution of KOH (7.80 g, 139.29 mmol) in ethanol (250 cm^3) under reflux. Then, benzyl bromide (25.80 g, 150.84 mmol) was added dropwise; the mixture was heated under reflux until no starting material was detectable (5 h.) by TLC (CH_2Cl_2/CH_3OH, 90/10). KBr was filtered off and washed with ethanol. The solution was evaporated and the residue was diluted in distilled water (100 cm^3). The mixture was acidified with aqueous HCl (2N) in order to obtain a clear solution. The solution was then washed with Et_2O (2x150 cm^3). Addition of NaOH 5N was added to precipitate the product. Solid **5** was filtered, dried then recrystallized in hexane (21.00 g, 84.26 mmol, 67 %)

Tf = 87°C (lit.89.5-90°C[18], 84-86°C[19])
^1H RMN ($CDCl_3$, 200 MHz) : δ 2.81 (s, 3H, Me) ; 5.46 (s, 2H, CH_2) ; 7.00 (dd, J_1=6.8 Hz, J_2=2.1Hz, 1H, H_7) ; 7.24 à 7.4 (m, 6H, H_{Ar}) ; 7.52 (m, 2H, H_{Ar}) ; 8.01 (d, J=8.4 Hz, 1H, H_4)
^{13}C RMN ($CDCl_3$, 200 MHz) : δ 25.69 (Me) ; 70.66 (CH_2) ; 110.40, 119.69, 122.38, 125.39, 126.67, 127.44, 128.37, 135.93 (CH_{Ar}) ; 127.59, 137.17, 139.95 ($C_{quat.Ar.}$) ; 153.70 (C_2=N) ; 157.99 (C_8-O)

2-Carboxaldehyde-8-benzyloxyquinoline : **6**[18,19]

Under nitrogen, compound **5** (20.00 g, 80.22 mmol) was added to a suspension of selenium oxyde (11 g, 100 mmol) in 150 cm^3 of dioxane and the mixture was stirred at 80°C for 2.5 h. The hot mixture was filtered on celite to remove black selenium. The solution was evaporated, and the residue was treated with acetone, red selenium was filtered off (millipore type HV 0.45 µm). Chromatography (silica gel, CH_2Cl_2/CH_3OH 99/1 v/v) afforded aldehyde **6** as a yellow solid (14.10 g ,53.55 mmol, 67%). This product can be recrystallised from acetone.

Tf = 94°C (lit. 93-94°C[18], 89-91°C[19])
IR (KBr) : 1670 cm^{-1} υ(C=O), 1090 cm-1 υ(C-O)
^1H RMN ($CDCl_3$, 200 MHz) : δ 5.49 (s, 2H) ; 7.15 (dd, J_1=7.4 Hz, J_2=1.5 Hz, H_7) ; 7.26 à 7.57 (m, 7H, $H_{5,6}$ + $H_{2',3',4',5',6'}$ of Bn) ; 8.06 (d, J=8.3 Hz, 1H, H_4) ; 8.27 (dd, J_1=8.3 Hz, J_2=0.8 Hz, H_3) ; 10.32 (d, J=0.8 Hz, 1H, CHO)
^{13}C RMN ($CDCl_3$, 200 MHz) : δ 70.88 (CH_2) ; 110.81, 117.64, 119.76, 126.93, 127.85, 128.53, 129.50, 137.09 (CH_{Ar}) ; 131.22, 136.36, 140.07 ($C_{quat. Ar}$) ; 151.34 (C_2=N); 154.95 (C_8-O); 193.75 (CHO)

2-Carboxy-8-benzyloxyquinoline : **7**

To aldehyde **6** (11.50 g, 43.68 mmol), dissolved in water (400 mL)-THF (200 cm^3), were successively added H_2NSO_3H (5.48 g, 56.44 mmol) and an aqueous solution of $NaClO_2$ (56.79 mmol in 40 cm^3 H_2O).The reaction was monitored by CCM (CH_2Cl_2/CH_3OH, 99/1). After 1 h., the mixture was concentrated under vacuum and extracted with ethyl acetate. This organic phase was extracted with an aqueous solution of NaOH 1N. Under cooling, the product precipitated upon addition of HCl 33% and was dissolved in CH_2Cl_2. The

solution was dried ($MgSO_4$) and the solvent was evaporated to give the pure product as a white powder (10.88 g, 38.87 mmol, 89 %).

Tf = 109°C
^1H RMN ($CDCl_3$, 200 MHz) : δ 5.30 (s, 2H, CH_2) ; 7.17 (dd, J_1=7.6 Hz, J_2=1.1 Hz, 1H, H_7) ; 7.30 à 7.59 (m, 7H, $H_{5,6}$ + $H_{2',3',4',5',6'\ of\ Bn}$) ; 8.21 (d, J=8.5 Hz, 1H, H_4) ; 8.29 (d, J=8.5 Hz, 1H, H_3) ; 10.08 (s large, 1H, OH)
^{13}C RMN (DMSO d_6, 200 MHz) : δ 69.68 (CH_2) ; 110.67, 119.11, 120.56, 127.06, 127.18, 127.78, 128.21, 136.59 (CH_{Ar}) ; 129.43, 136.33, 138.60 ($C_{quat.\ Ar}$) ; 146.91 (C_2=N); 154.10 (C_8-O); 165.82 (COOH)

Tris-N-(2-aminoethyl-[8-benzyloxyquinoline-2-carboxamido])amine : **8**

To a suspension of **7** (5.00 g, 17.86 mmol) in 200 cm^3 THF, under nitrogen, was added 1,1'-carbonyldiimidazole (3.19 g, 19.67 mmol). TREN (0.87 g, 5.95 mmol) in 100 cm^3 THF was added dropwise at reflux for 1h.. The mixture was stirred for 18 h. at 50°C. The solvent was eliminated and the residue treated with 200 cm^3 of $CHCl_3$. The solution was washed with 100 cm^3 of saturated NH_4Cl then brine (100 cm^3), then dried ($MgSO_4$). The produit was purified by chromatography (silica gel, CH_2Cl_2/CH_3OH 98/2). The podand was obtained as a white solid (3.04 g, 3.27 mmol, 55 %).

Tf = 78°C
IR (KBr) : 3460-3360 cm^{-1} υ(N-H) ; 1650 cm^{-1} υ(C=O) ; 1090 cm^{-1} υ (C-O).
^1H RMN ($CDCl_3$, 200 MHz) : δ 2.70 (t, J=6.3 Hz, 6H, CH_2) ; 3.41 (dt, J_1=6.0 Hz, J_2=6.0 Hz, 6H, CH_2) ; 5.18 (s, 6H, $CH_{2\ Bn}$) ; 6.93 (d, J=7.5 Hz, 3H, H_7) ; 7.19 à 7.58 (m, 21H, H_5+H_6+H_{Ph}) ; 7.88 + 7.98 (AB, J_{AB}=8.5 Hz, 6H, H_3+H_4) ; 8.38 (t, J=5.4 Hz, 3H, NH).
^{13}C RMN ($CDCl_3$, 200 MHz) : δ 37.43 (CH_2) ; 52.59 (CH_2) ; 70.73 (CH_2) ; 109.81, 118.95, 119.60, 127.64, 127.79, 128.05, 128.57 136.49 (CH_{Ar}) ; 130.06, 136.60, 138.27 ($C_{quat.}$) ; 148.71 (C_2) ; 154.59 (C_8) ; 164.69 (C=O)
SM FAB(+) (thioglycerol+glycerol): $(M+H)^+$ 930; 840, 638, 305, 215, 144
FAB(-) : 929 ; 839, 748, 658, 213, 199, 144.
Anal. : Calcd (Found) for $C_{57}H_{51}N_7O_6$, CH_3OH: C, 72.39 (72.26); H, 5.77 (5.61); N, 10.20 (10.33)

Tris-N-(2-aminoethyl-[8-hydroxyquinoline-2-carboxamido])amine: **9 (N-Trenox)**

To a solution of **8** (0.9 g, 0.97 mmol) in CH_2Cl_2 (100 mL) under N_2 at 0°C, was added BBr_3 (14.5 cm^3 of a 1M solution in CH_2Cl_2). The reaction mixture was allowed to warm to ambient temperature and stirred for 15 h. Methanol (150 cm^3) was added and the mixture stirred for 2 h. The resulting solid was filtered and taken up with aqueous NaOH 1N (50 cm^3) and precipated again under cooling by addition of HCl 33% until pH = 6-7. Filtration and drying afforded, a white solid (0.51 g, 0.78 mmol, 80%)

Tf = 218-220°C (lit.[12] 222-223°C)

IR (KBr) : 3600-3100 cm^{-1} υ(O-H) ; 1650 cm^{-1} υ(C=O) ; 1220 cm^{-1} υ(C-O)

^1H RMN (DMSO d_6, pH ≈ 7 , 200 MHz) : δ 2.89 (t, J=6.7 Hz, 6H, CH_2) ; 3.54 (br. dt 6H, CH_2) ; 7.14 (dd, J_1=7.5 Hz, J_2=1.0 Hz, 3H, H_7) ; 7.44 (dd, J_1=7.5 Hz, J_2=1.0 Hz, 3H, H_5) ; 7.54 (dd, $J_1 \approx J_2 \approx$7.5 Hz, 3H, H_6) ; 8.09 + 8.44 (AB, J_{AB}=8.5 Hz, 6H, H_3+H_4) ; 9.64 (br.t J=5.7 Hz, 3H, NH) ; 10.05 (s, 3H, OH)

^{13}C RMN (DMSO d_6, 200 MHz, 2D "Delta hetero") : δ 37.45 (CH_2) ; 53.66 (CH_2) ; 111.44 (C_7), 117.47 (C_5), 118.69 (C_3), 129.27 ($C_{quat.\ cycl\ (10)}$), 129.37 (C_6) ; 136.33 ($C_{\ quat.\ cycl.(9)}$) ; 137.59 (C_4) ; 147.41 (C_2=N) ; 153.50 (C_8-O) ; 163.75 (C=O)

SM (thioglycerol+glycerol) FAB(+): $(M+H)^+$ 660 ; 215, 144

FAB(-): 658, 213, 199, 144

9,2HCl : FAB(+) (NBA) : $(M+H)^+$ 732, 695, 660

Anal. : Calcd (Found) for $C_{36}H_{33}N_7O_6$, HCl, H_2O: C, 60.57 (60.72); H, 5.09 (4.90); N, 13.74 (13.14)

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